

論文の要旨 (Thesis Summary)

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論文題目(Thesis Title)

Studies on bioethanol production by recombinant thermophilic acetogen *Moorella thermoacetica*
(遺伝子組換え好熱性ホモ酢酸菌によるバイオエタノール生産に関する研究)

Chapter 1. Introduction

The high energy demand has been met by fossil fuels. It has been recognized that it will not only deplete the world's oil reservoir, but also has a serious impact on the environment, leading to increased health risk and global climate change which makes the need for alternative fuel solutions significant. Renewable energy is classified as energy which regenerates within a short time. It generates less greenhouse gas emissions than fossil fuels and otherwise pollutes less. Developing ethanol as fuel would require lignocellulose as a feedstock. Ethanol can be produced by many microorganisms from various types of substrates. Thermophilic bacteria are capable to produce ethanol. The greatest advantage of the application of thermophilic bacterial strains in biofuel production is the capability of hydrolyzing components of biomass to produce bioethanol. Alternatively, metabolic engineering has produced high yield ethanol fermentation in model organisms that can be genetically manipulated. *Moorella thermoacetica* is a considerable as an alternative genetically engineered host for efficient production of ethanol from lignocellulosic sugars.

Chapter 2. Thermophilic ethanol fermentation by genetically engineered *Moorella thermoacetica*

A transformant of *M. thermoacetica* was constructed for thermophilic ethanol production from lignocellulosic biomass by deleting two phosphotransacetylase genes, *pdul1* and *pdul2*, and introducing the native aldehyde dehydrogenase gene (*aldh*) controlled by the promoter from the glyceraldehyde-3-phosphate dehydrogenase gene. The transformant showed tolerance to 540 mM ethanol and fermented sugars including fructose, glucose, galactose and xylose to mainly ethanol. In a mixed-sugar medium of glucose and xylose, all of the sugars were consumed to produce ethanol at the yield of 1.9 mol/mol-sugar. The transformant successfully fermented sugars in hydrolysate prepared through the acid hydrolysis of lignocellulose to ethanol.

Chapter 3. Ethanol yield and sugar usability in ethanol production from lignocellulose hydrolysate by genetically engineered *Moorella thermoacetica*

Bioconversion from inexpensive renewable resource, such as biomass, to liquid fuel is one of the promising technologies to reduce the use of petroleum. I demonstrated the genetically engineered *M. thermoacetica* could produce ethanol from the lignocellulosic feedstock in the chapter 2. However, it was still unclear which carbon source in the substrate was preferentially consumed to produce ethanol. To identify the hierarchy of the sugar utilization during ethanol fermentation of this strain, I analyzed the sugar composition of lignocellulosic feedstock, and consumption rate of sugars during the fermentation process. The hydrolysates after acid

pretreatment and enzymatic saccharification contained glucose, xylose, galactose, arabinose, and mannose. Time course data suggested that xylose was the most preferred carbon source among those sugars during ethanol fermentation. Ethanol yield was 0.40 ± 0.06 and 0.40 ± 0.12 g/g-total sugar, from lignocellulosic hydrolysates of Japanese cedar (*Cryptomeria japonica*) and rice straw (*Oryza sativa*), respectively. The results demonstrated that the genetically engineered *M. thermoacetica* is a promising candidate for thermophilic ethanol fermentation of lignocellulosic feedstocks, especially hemicellulosic sugars.

Chapter 4. Conclusion

In the study, I constructed the transformant of *M. thermoacetica* for thermophilic ethanol production from lignocellulosic biomass by deleting phosphotransacetylase genes and introducing the aldehyde dehydrogenase gene. The transformant successfully fermented not only various sugars included in lignocellulose but also actual lignocellulose hydrolysates to ethanol. Ethanol yield reached 0.40 g/g-total sugar, from both lignocellulose hydrolysates of Japanese cedar (*Cryptomeria japonica*) and rice straw (*Oryza sativa*). The present findings demonstrated that thermophilic fermentation by genetically engineered *M. thermoacetica* is feasible for producing ethanol from lignocellulosic biomass.